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(54) **Title:** DISINFECTANT AND SPORICIDAL COMPOSITION CONTAINING A LOW CONCENTRATION OF GLUTARALDEHYDE FOR CLEANING AND DISINFECTION OF MEDICAL, VETERINARY OR INDUSTRIAL EQUIPMENT

(57) **Abstract:** Disinfectant and sporicidal composition containing a low concentration of glutaraldehyde for cleaning and disinfection of medical, veterinary or industrial equipment in short times.

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Disinfectant and sporicidal composition containing a low concentration of glutaraldehyde for cleaning and disinfection of medical, veterinary or industrial equipment.

Field of the invention

The present invention relates to a disinfectant and sporicidal composition containing a low concentration of glutaraldehyde, which composition is useful for cleaning and disinfecting materials used in medical, veterinary or industrial environments, including the food industry. The composition of the present invention is highly effective in killing a wide range of bacteria, viruses, fungi and spores.

Background art

Traditionally, 2% alkaline glutaraldehyde has been used as a high-level disinfectant (HLD). Throughout the present document, the definitions of low, intermediate and high level disinfectant follow the criteria set out in "Guideline for Disinfection and Sterilization in Healthcare Facilities, 200" updated May 2019, by William A. Rutala, Ph.D, M.P.H., David J. Weber, M.D., M.P.H., and the Healthcare Infection Control Practices Advisory Committee (HICPAC), that can be found in https://www.cdc.gov/infectioncontrol/guidelines/disinfection/, as explained below:

- High level disinfectants: Compounds that kill all microorganisms except large numbers of bacterial spores at short exposure periods (around 20 minutes for 2% glutaraldehyde);
- Intermediate level disinfectants: They may be cidal for mycobacteria, vegetative bacteria, most viruses, and most fungi but do not necessarily kill bacterial spores;
- Low level disinfectants: They can kill most vegetative bacteria, some fungi, and some viruses in a practical period of time (≤10 minutes).

Traditionally, 2% alkaline glutaraldehyde compositions are usually activated with bicarbonate at a concentration of approximately 0.3%, resulting in a HLD that has only been sporicidal at very long times (3-8 h) and has also failed in the disinfection of *M. avium intracellulare* unless the compositions are left to act for 45 minutes, more than twice the time normally recommended for high-level disinfection with 2% glutaraldehyde, which is 20 minutes.

Moreover, these compositions, due to their high concentration of glutaraldehyde, are toxic or respiratory irritant, since at 2% they produce vapours requiring forced ventilation to prevent their accumulation, which is not always the case in the rooms where instruments are disinfected. On the other hand, these compositions are not aggressive on hospital equipment, but if the latter contains traces of organic matter, that organic matter becomes fixed to the substrate and, on the one hand, alters the instruments and, on the other hand, causes a reduction in the diameter of narrow lumens, to the point of requiring repair of the fomite.

Attempts have been made to reduce the toxicity of glutaraldehyde by reducing its concentration and combining it with other substances that amplify its action such as phenolic derivatives, so that its concentration has been diluted to 0.25 % or even 0.13%, but its efficacy was insufficient against mycobacteria to be considered HLD, so it is not recommended at concentrations lower than 1%, and this concentration still produces toxic effects.

In the prior art it is possible to find documents such as US5863547, which describes compositions with glutaraldehyde in a concentration closer to that traditionally used, namely 1.0% to 2.5% in combination with phosphate salts.

There are also other documents such as CN105394034A and CN101579330A which disclose high-level aqueous disinfectant compositions containing higher glutaraldehyde concentrations of between 1% and 1.5%.

US3968250 describes aqueous sporicidal and disinfectant compositions comprising glutaraldehyde in theoretic concentrations from 0.02% to 5%, an

alcohol, a sodium bicarbonate buffer and a non-ionic or anionic compound which is an ethoxylate of linear isomeric alcohols in a concentration between 0.01% and 1%. These compositions are used in conjunction with ultrasonic irradiation over a wide frequency range (from 10 to 850 kHz). The latter complicates and greatly reduces their use in various settings, hospital or otherwise, and does not eliminate the toxicity of glutaraldehyde, since in the examples it is preferably used at concentrations higher than 1%, typically 2%.

CN111955488A describes aqueous disinfectant compositions comprising 0.1 to 1.5% glutaraldehyde, isopropanol, a salt such as sodium nitrite, isopropyl alcohol and the compounds didecyl-dimethyl-benzyl-ammonium chloride and didecyl-dimethyl-ammonium chloride (chlorinated quaternary ammoniums).

RU 2 395 962 C1 discloses disinfectant and sporicidal compositions comprising glutaraldehyde 0.5% and alkyl dimethylbenzylammonium chloride 10.0%, a chlorinated ammonium salt. The compositions disclosed in this document include a high content of hydrogen peroxide, that can antagonize the reducing effect of the glutaraldehyde. In addition, the compositions disclosed in this document do not contain any alkaline or alkaline-earth salts.

CN 115 530 166A discloses animal disinfectant compositions containing high glutaraldehyde concentrations of 1-15% and decyl ammonium bromide 1-10%. Again, the compositions disclosed in this document do not contain any alkaline or alkaline-earth salts.

CN 106 804 620A discloses a disinfectant for tools for silkworm rearing liquid containing glutaraldehyde (0.6-1.1%) and an undefined quaternary ammonium salt (0.2-0.7%), plus very high ethanol concentrations (30-40%) and a strong oxidant like an iodophor, that can antagonize the effect of the glutaraldehyde. Finally, again, the compositions disclosed in this document do not contain any alkaline or alkaline-earth salts.

CN 111 096 322A discloses disinfectant compositions comprising either extremely low (0.0025 to 0.025% v/v) or rather high (1 to 1.5%)

glutaraldehyde concentrations, very high ethanol concentrations (5-65%), and didecyldimethylammonium bromide. Here again, the compositions disclosed in this document do not contain any alkaline or alkaline-earth salts.

Finally, CN 107 509 740A discloses a disinfectant for veterinary purposes in clinics comprising glutaraldehyde 0.4 g/L-0.6 g/L, OTAC (octadecyl trimethyl ammonium chloride, a chlorinated ammonium salt) 0.4g/ L-0.6g/L, plus chlorhexidine acetate. The concentration of glutaraldehyde concentration of these compositions is rather low, and the chlorhexidine acetate can produce problems of aggressivity towards the materials to be disinfected. Also, the compositions disclosed in this document do not contain any alkaline or alkaline-earth salts.

Therefore, there still exist a need to provide disinfectant and sporicidal compositions with glutaraldehyde at low concentration that overcome the disadvantages of those already known in the prior art, and particularly that exhibit a high level disinfectant activity while at the same time have their toxicity towards humans as well as their aggressivity towards the material markedly reduced.

Consequently, the technical problem to be solved by the skilled person can be considered as how to provide disinfectant and sporicidal compositions having a high efficacy accompanied by a very low aggressivity towards both the materials and the human's health, which is something that cannot be found presently in the market of bactericidal and sporicidal compositions.

The inventor of the present invention has found that it is possible to achieve a sporicidal HLD activity in very short times, of the order of 10-15 minutes at a glutaraldehyde concentration of only 0.25%, in combination with a very low toxicity for the human health and aggressivity to the material disinfected, by providing aqueous compositions containing glutaraldehyde at a concentration between 0.1% w/v and 1% w/v, which is activated with the following three components:

- at least one non-chlorinated cationic quaternary ammonium compound selected from didecyl-methyl-polyoxy-ethyl-ammonium propionate at a concentration of 0.1-5% v/v, or tetradecyl-trimethyl-ammonium bromide at a concentration of 0.1-5% w/v;
- at least one alcohol selected from the group consisting of n-propanol, isopropanol and ethanol, individually or in any combination thereof, in a concentration of 6-20% v/v; and
- at least one alkaline or alkaline earth salt in a concentration of between 0.05% w/v and 0.5% w/v.

According to the inventor's findings and in line with the results of the below-explained experimental tests, the combination of these components shows a clear synergistic effect, having demonstrated a clear efficacy against mycobacteria and other more sensitive (hospital) bacteria, which are found on rough-surfaced germs (endodontic files). The compositions of the invention are completely effective within 10 minutes and are not aggressive on metal even after 7 days of immersion.

Detailed description of the invention:

The disinfectant and sporicidal compositions of the present invention are aqueous compositions containing glutaraldehyde at a concentration between 0.1% w/v and 1% w/v, which is activated with the following three components:

- at least one non-chlorinated cationic quaternary ammonium compound selected from didecyl-methyl-polyoxy-ethyl-ammonium propionate at a concentration of 0.1-5% v/v, or tetradecyl-trimethyl-ammonium bromide at a concentration of 0.1-5% w/v;
- at least one alcohol selected from the group consisting of n-propanol, isopropanol and ethanol, individually or in any combination thereof, in a concentration of 6-20% v/v; and

- at least one alkaline or alkaline earth salt in a concentration of between 0.05% w/v and 0.5% w/v.

In embodiments, the glutaraldehyde is present in a concentration of between 0.1% and 0.5% w/v, or about 0.25% w/v, or about 0.13%.

In embodiments, the non-chlorinated cationic quaternary ammonium compound is didecyl-methyl-polyoxy-ethyl-ammonium propionate and is present is at a concentration of 1-3% v/v.

In embodiments, the non-chlorinated cationic quaternary ammonium compound is tetradecyl-trimethyl-ammonium bromide and is present at a concentration of 0.1-1% w/v.

In embodiments, the alcohol is present in a concentration range of 6-20% v/v, more preferably around 6% v/v.

In embodiments, the alkaline or alkaline-earth salt is sodium or potassium carbonate or bicarbonate, individually or in combination, and is present in a concentration range of between 0.05% w/v and 0.5% w/v.

Water is used as a solvent, and is added in sufficient quantity (q.s.) to reach 100% vol.

In the experiments carried out, the compositions of the invention have shown the highest possible sporicidal HLD level using spores of *B atrophaeus 3M* in very short times of exposure, 10-15 minutes, at a glutaraldehyde concentration of only 0.25%. For this purpose, different activators are used which have demonstrated a synergistic effect and non-aggressiveness on the material to be disinfected with glutaraldehyde, an effect that has not been demonstrated by other activators in the comparative tests made. Their efficacy against mycobacteria and other bacteria (hospital bacteria) more sensitive to disinfectants, which are on rough germs (endodontic files), has

also been proven. They are completely effective in 10 minutes and are not aggressive on metal or in 7 days of immersion.

Accordingly, in a first aspect, the invention is directed to a disinfectant and sporicidal composition comprising:

- glutaraldehyde at a concentration between 0.1% w/v and 1% w/v;
- at least one non-chlorinated cationic quaternary ammonium ammonium compound selected from didecyl-methyl-polyoxy-ethyl-ammonium propionate at a concentration of 0.1-5% v/v, or tetradecyl-trimethyl-ammonium bromide at a concentration of 0.1-5% w/v;
- at least one alcohol selected from the group consisting of n-propanol, isopropanol and ethanol, individually or in any combination thereof, in a concentration of 6-20% v/v;
- at least one alkaline or alkaline earth salt in a concentration of between 0.05% w/v and 0.5% w/v, and
- water, q.s. to 100% vol.

In a second aspect, the invention is directed to a method for cleaning and disinfecting materials for medical or veterinary use using the above-described composition, comprising diluting the composition with water to the desired concentration if required, applying the diluted composition to the surface and/or ducts of the material to be disinfected, leaving the composition on the surface for a predetermined time, rinsing the material with water, and drying the material.

In a third aspect, the invention is directed to the use of the above-mentioned composition in the high-level disinfection of medical or veterinary instruments or in the medium/low-level disinfection of surfaces or medical or veterinary instruments or even in the food industry.

In a fourth aspect, the invention is directed to a kit for the *in situ* reconstitution of the above-mentioned disinfectant and/or sporicidal composition, wherein the glutaraldehyde is in a first container, the alcohol and the ammonium compound are in a second container, and the salt or salts in a third container.

In a fifth aspect, the invention is directed to a process for the *in situ* reconstitution of a disinfectant and/or sporicidal composition, as defined above, comprising the step of providing the glutaraldehyde, alcohol and/or salt at a concentration higher than that necessary to generate the reconstituted composition, and then diluting them, in situ, to the required concentration.

Experimental tests

Materials and methods

For the present experimental tests, a base formulation with 0.25% glutaraldehyde (1/100 dilution of 1 ml of 25% Panreac glutaraldehyde) was used, to which one of the following combinations of activators was added:

Activator s+t8+a

Activator s+B+a

Activator s+T+a

Activator s+Q+a

Activator s+E+a

Activator s+D+a

s+I+a activator

The rest, up to 100 vol%, is sterile distilled water.

where:

"s" is the alkaline salt, that is a mixture of sodium bicarbonate 0.5 % w/w and potassium carbonate 0.18 % w/w,

"a" is the alcohol, that is n-propanol, 6 ml per 100 ml (%v/v),

"T8" is tetranil T80 (coco-alkyl-alkyl-dimethyl-benzyl-ammonium chloride), 1% v/v,

"B" is Bardap 26 (didecyl-methyl-polyoxy-ethyl-ammonium propionate), 1% v/v,

"T" is Tetradecyl-trimethyl ammonium bromide, 0.125% w/v,

"Q" is alkylpropylenediamine-guanidinoacetate, 1% v/v,

"E" is Emal 40 (triethanolamine lauryl sulfate), 2% v/v,

"D" is dodecylsulphate, 0.1% w/v,

"I" is copolymer of ethylene oxide and propylene oxide, 2% v/v,

With regard to the salt, previous experimental tests carried out by the inventor have shown that, with the double salt sodium bicarbonate + potassium carbonate, the results are better and the pH is more stable than with a single salt like sodium bicarbonate alone.

With regard to the alcohol, in previous experimental tests carried out by the inventor, the alcohols that gave the best results are 3-carbon alcohols, such as isopropyl and n-propyl alcohol.

The above-explained formulations were used on:

- Spores of *B atrophaeus* (formerly B subtilis), commercial (3M).

- *M fortuitum* ATCC.

- *P aeruginosa* obtained from an ICU patient.

Initially, the sporicidal effect on commercial spores (3M) and the bactericidal effect on material with a rough surface (endodontic files) or smooth surface (glass) was determined in order to check their efficacy on mycobacteria and other more sensitive bacteria. Its aggressiveness on scalpel with human blood at different times was also analyzed. All these techniques are described in several works by Dr. Herruzo, Dr. Vizcaino et al. between 1999-2004.

Microbicidal efficacy tests

Figures 1 and 2 show the difference in efficacy between classical glutaraldehyde (2% alkaline glutaraldehyde) and solutions with lower glutaraldehyde concentrations that have been activated with the activator s+T+a, hereafter referred to as Activator1 (Acti1). The tests in Figure 1 show that sporicidal action can be achieved in 5 minutes with 1% glutaraldehyde (G+1%), or just 15 minutes if only 0.25% (G+0.25%) is used. With mycobacteria (Figure 2) the efficacy is even higher despite being on a rough germ carrier, as 1% glutaraldehyde (G+1%) requires only 3 minutes to destroy the entire mycobacterial inoculum, 0.5% (G+0.5%) requires 5 minutes and 0.25% (G+0.25%) requires just 10 minutes.

With *P. aeruginosa* the result is even better, as it destroys the entire inoculum in only 5 minutes with any of the three concentrations of glutaraldehyde tested (1%, 0.5% and 0.25%).

Figure 3 shows the sporicidal results obtained when comparing a solution containing just 0.25% glutaraldehyde alone (i.e. without activator), activator Acti1 (=s+T+a) alone (i.e. without glutaraldehyde) and activator Acti2 (=s+B+a) alone (i.e. without glutaraldehyde). The sporicidal effect is expressed as the $\log_{10}$ reduction of *B atrophaeus* spores in 15 minutes and the bactericidal effect (on rough germs) as the $\log_{10}$ reduction of *M. fortuitum* in 10 minutes and of P. aeruginosa in 5 minutes.

These tests clearly show that the glutaraldehyde 0.25% alone (without activator), and each of activators Acti1 or Acti2 alone (without glutaraldehyde)

produce a very poor sporicidal effect. However, when the glutaraldehyde 0.25% is combined with either activators Acti1 or Acti2 (which already contain the mix of salts "s" and the alcohol "a"), the sporicidal effect results multiplied by a factor between 4 and 5. For the inventor, this enormous increase in sporicidal effect is something completely surprising and unexpected in this technical field.

Figure 4 shows the bactericidal effect for the same compositions tested in Figure 3. Again, the solution containing just 0.25% glutaraldehyde alone (without activators) or the solutions containing just the activators Acti1 or Acti 2 (without glutaraldehyde) produce hardly any measurable microbicidal effect, whereas the combinations of 0.25% glutaraldehyde with either activator Acti1 or Acti2 destroy more than 5 $\log_{10}$ (both spores in 15 minutes and mycobacteria in 10 minutes), and this maximum efficacy occurs even when a very low glutaraldehyde concentration, much lower than the concentration commonly believed as necessary to achieve a good sporicidal or bactericidal efficacy, is used. Again, this enormous increase in sporicidal effect is something completely surprising and unexpected in this technical field.

In view of the high antimicrobial activity obtained, the sporicidal effect was tested again with *B. atrophaeus*, but in a shorter exposure time (10 min) and only with glutaraldehyde 0.25%, with the two types of preferred activators, namely Act2 that contains the non-chlorinated ammonium compound "B", and Act1 that contains the non-chlorinated ammonium compound "T". The results showed that activator Acti1 (s+T+a) failed to reduce the 5 $\log_{10}$ of *B atrophaeus* spores in only 10 min, while Acti2 (s+B+a) did. Therefore, the activator Acti1 (Gluta 0.25% + Acti1) can be allocated to the indication of 15 min exposure, whereas for Acti2 (Gluta 0.25% + Acti2) the indication can be reduced to only 10 min.

The results also show that classic glutaraldehyde alone (Gluta 0.25%), or the two activators alone (Acti1 and Acti2), do not achieve to pass 2 $\log_{10}$ of spores

reduced in 15 min, and their efficacy against *Mycobacteria or P aeruginosa*, in shorter times, is also scarce.

Figure 5 shows the sporicidal effect of compositions containing each one of the following components:

- Gluta 0.25%: Composition containing Glutaraldehyde 0.25% alone;
- Gluta 0.25% + Acti-a: Composition containing just Glutaraldehyde 0.25% plus the alcoholic component "a", i.e., n-propanol, 6 ml per 100 ml (%v/v), but without both the ammonium component and the salt component "s";
- Gluta 0.25% + Acti-B: Composition containing just Glutaraldehyde 0.25% plus the ammonium component "B", i.e., didecyl-methyl-polyoxy-ethyl-ammonium propionate, but without both the alcohol component "a" and the salt component "s";
- Gluta 0.25% + Acti-s: Composition containing just Glutaraldehyde 0.25% plus the salt component "s", i.e., a mixture of sodium bicarbonate 0.5% w/w and potassium carbonate 0.18% w/w, but without both the ammonium compound and the alcohol component "a";
- Activ a+B+s: Composition containing just components "a", "B" and "s" but without glutaraldehyde; and
- Gluta 0.25% + acti-a+B+s: Composition containing Glutaraldehyde 0.25% with the components "a", "B" and "s".

The results show that the composition containing glutaraldehyde 0.25% alone, the compositions containing glutaraldehyde 0.25% with either component "a", component "B" or component "s" taken each one individually together with the glutaraldehyde 0.25%, or the composition containing components "a", "B" and "s" in admixture but without glutaraldehyde 0.25%, show a very poor sporicidal effect. It is only when the glutaraldehyde 0.25% is combined with the three components "a", "B" and "s" when the sporicidal effect becomes unexpectedly multiplied. Again, in the inventor's view, this effect is completely surprising and unexpected in the present technical field.

With the activator Acti2 (= s+B+a) in combination with 0.25% glutaraldehyde, a further step was also attempted: the destruction of more than 10^6 *B atrophaeus* spores, i.e. the threshold at which we define "sterilisation" by chemical methods. For this purpose, 3M commercial spores, which have a certified load of 3-5 million *B. atrophaeus* spores, were used and introduced into the disinfectant. After 15 to 20 min (50% or 100% longer than the time for the sporicidal action described above) they were inhibited in a suitable inhibitor and, instead of shaking to dislodge the surviving spores, the germs were placed in culture broth (Nutrient-Broth, Difco) for 7 days. If one or more spores had survived, growth would appear in the broth, so the absence of such growth would indicate that no spores survive, i.e. the level of spore destruction required for sterilisation is achieved.

The result of this test is also excellent. In only 15 minutes, the spore load of the commercial germ carrier was completely eliminated, so it can be concluded that sterilisation of the material immersed in this diluted solution of glutaraldehyde (0.25%) with the latter activator is achieved in this time.

Aggressivity tests

Finally, the inventor carried out additional comparative experimental tests shown in Fig. 6, where the combined action of the microbicidal efficacy and the aggressivity effect of the two preferred quaternary ammonium compounds T and B were tested and compared with the other quaternary ammonium compounds T8, Q, D, E or I. To that end, in order to determine the combined action of the microbicidal effect and the aggressivity effect of the compounds under testing, the inventor developed a "synthetic score", calculated as follows:

The microbicidal efficacy, measured according to the logarithmic reduction (log_{10}) of B subtilis spores, is scored from 0 to 6 marks according to the following table:

0 - 0.1 log10 = 0 marks

0.1 - 1 log10 = 1 mark
1.1 – 2 log10 = 2 marks
2.1 – 3 log10 = 3 marks
3.1 – 4 log10 = 4 marks
4,1 – 5 log10 = 5 marks
5,1 - ≥6 log10 =6 marks

The aggressivity on the material was evaluated by the alteration of the surface of a scalpel with blood, after 72 hours of immersion. This is scored from 0 to 4 marks according to the following table:

Alteration of the entire surface of the blade =	1 mark
Alteration of several areas on the surface of the blade =	2 marks
Alteration of one area on the surface of the blade =	3 marks
No alteration on the surface of the blade =	4 marks

As can be seen, in the final score, the part related to the microbicidal activity has a somewhat higher weight (60%) than the aggressivity activity (40%), in accordance with the inventor's criteria. As it is clear, the highest number of marks according to this synthetic score, i.e. 10 marks, correspond to the highest microbicidal efficacy combined with the lowest aggressivity to the material.

The results of the comparative experimental tests carried out comparing the different compounds tested shown in Figure 6 demonstrate that the combined non-chlorinated ammonium compounds T and B have an excellent result in the combined activity of the microbicidal test and the aggressivity test, perhaps followed by the also non-chlorinated ammonium compound E.

Conclusions

In conclusion, compositions containing just glutaraldehyde 0.25% show a poor sporicidal and bactericidal effect. Similarly, compositions containing just the component "a" (alcohol) or the component "s" (salt) even if accompanied by

glutaraldehyde 0.25%, also do not show any sporicidal or bactericidal effect in the absence of the non-chlorinated ammonium compound. In addition, compositions containing just the ammonium B or the ammonium T alone, accompanied by either glutaraldehyde 0.25% or by the components "a" or "s", again do not show any noticeable sporicidal or bactericidal effect. Even more, mixtures of glutaraldehyde 0.25% with whether component "s", component "a" or the ammoniums T or B alone, likewise do not show any substantial sporicidal effect. And furthermore, mixtures of components "a", "B" and "s" again do not show any noticeable sporicidal effect in the absence of glutaraldehyde. It is only when the glutaraldehyde 0.25% is combined with the alcohol "a", the salt component "s" and the non-chlorinated ammoniums T or B, a very outstanding and surprising sporicidal effect is observed, despite the low glutaraldehyde concentration used. And these compositions have the additional advantage that, having a reduced glutaraldehyde concentration, their aggressivity towards the materials and the health of the persons handling these compositions is substantially reduced or even annulled, which is a very important advantage for this type of compositions that require a substantial manual handling.

For surface disinfection, where the evaporation of glutaraldehyde may be higher, it may be useful to use an even lower concentration of this product in order not to contaminate the environment. For this reason, the efficacy has been tested, under these conditions, with different activators, the most effective being s+B+a, which achieves 3.6 $\log_{10}$ reduction of *B. atrophaeus* spores with only 0.13% aldehyde (1/2 of the previous formula, used as HLD) after a 30-minute contact, i.e. a similar efficacy to hypochlorite 4000 ppm, but without the toxicity and aggressiveness to metal of the latter. If mycobacteria or bacteria of hospital origin on glass germ carriers (surface model) are used, this synergistic combination completely destroys the inoculums of all of them in 10 minutes.

As a result of all the above, in the inventor's view, the compositions claimed show a substantial unexpected and advantageous effect, which could have not

been deduced in an obvious manner from the prior art and that also could not have been obtained just by routine experimentation in view of the vast possibilities of quaternary ammonium compounds available at the reach of the skilled person that could potentially be tried, and that in the great majority of cases are chlorinated ammonium compounds, as opposed to the non-chlorinated ammonium compounds used in the present invention.

The disinfectant and sporicidal composition of the present invention can be used for high-level cleaning and disinfection of materials used in medical or veterinary environments, including but not limited to, surgical instruments, medical devices, hospital equipment, veterinary equipment and laboratory equipment, as the composition is effective in killing a wide range of microorganisms, even those that are resistant to other disinfectants, including bacteria, viruses, fungi and spores.

The composition may also contain other ingredients, such as other compounds, chelating agents and pH adjusters, to improve its cleaning and disinfecting properties. The composition may be prepared as a liquid, aerosol or foam.

The composition can be obtained at a higher concentration, but can be diluted with water to the desired concentration, depending on the application. The diluted composition can be applied to the surface of the material as a medium-level disinfectant and left for e.g. 30 min to allow the removal of any micro-organisms. The material can be rinsed with water and dried.

The composition can also be used as an immersion solution (of surfaces or internal instrument ducts), where the material is soaked in the solution for a specified period of time before being removed and allowed to dry.

The composition of the present invention offers several advantages over existing disinfectants and sporicidal compositions. The low concentration of glutaraldehyde used in the composition reduces the risk of toxicity and skin

or airway irritation to personnel handling it, making it safer for use by medical and veterinary professionals. But despite this dilution, the composition is also highly effective in killing a wide range of microorganisms, including those that are resistant to other disinfectants, making it an ideal choice for use in medical, veterinary and industrial facilities.

In addition, the compound used in the composition helps to improve the wetting and penetration of the solution into the material, thus increasing its effectiveness in killing micro-organisms.

In conclusion, the disinfectant and sporicidal composition of the present invention provides an effective and safe means of cleaning and disinfecting materials used in medical or veterinary environments or even in the food industry. The low concentration of glutaraldehyde used in the composition, together with the presence of a non-chlorinated ammonium compound, makes the composition less toxic and more effective than conventional disinfectants. The composition can be used as a dipping solution.

CLAIMS

1. A disinfectant and sporicidal composition comprising:

- glutaraldehyde in a concentration of between 0.1% w/v and 1% w/v;
- at least one non-chlorinated cationic quaternary ammonium compound selected from didecyl-methyl-polyoxy-ethyl-ammonium propionate in a concentration of 0.1-5% v/v, or tetradecyl-trimethyl-ammonium bromide in a concentration of 0.1-5% w/v;
- at least one alcohol selected from the group consisting of n-propanol, isopropanol and ethanol, individually or in any combination thereof, in a concentration between 6-20% v/v;
- at least one alkaline or alkaline earth salt in a concentration between 0.05% w/v and 0.5% w/v; and
- water, q.s. to 100% vol.

2. A disinfectant and sporicidal composition according to claim 1, wherein the glutaraldehyde is in a concentration of between 0.1% w/v and 0.5% w/v.

3. A disinfectant and sporicidal composition according to claim 2, wherein the glutaraldehyde is in a concentration of 0.25% w/v.

4. A disinfectant and sporicidal composition according to claim 2, wherein the glutaraldehyde is in a concentration of 0.13% w/v.

5. A disinfectant and sporicidal composition according to any one of claims 1 to 4 above, wherein the alcohol is in a concentration of 6 % v/v.

6. A disinfectant and sporicidal composition according to any one of claims 1 to 5 above, wherein the alkaline or alkaline earth salt is sodium or potassium carbonate or bicarbonate, in any combination thereof.

7. A disinfectant and sporicidal composition according to claim 6, wherein the alkaline or alkaline earth salt is in a concentration of between 0.05% w/v and 0.5% w/v.

8. A disinfectant and sporicidal composition according to any one of the preceding claims, wherein the didecyl-methyl-polyoxy-ethyl-ammonium propionate is in a concentration of 1-3% v/v.

9. A disinfectant and sporicidal composition according to any one of the preceding claims, wherein the tetradecyl-trimethyl-ammonium bromide is in a concentration of 0.1-1% w/v.

10. A method for cleaning and disinfecting materials for medical, veterinary or industrial use using the composition of any one of claims 1 to 9, comprising diluting the composition with water to the desired concentration if required, applying the composition to the surface of the material to be disinfected, leaving the composition on the surface for a predetermined time, rinsing the material with water, and drying the material.

11. The method of claim 10, wherein the material is a surgical instrument, endoscope, dental equipment, or other medical or veterinary material.

12. Use of the composition of any one of claims 1-9 above for high-level disinfection of medical or veterinary instruments or for medium or low-level disinfection of sanitary surfaces or medical or veterinary instruments.

13. Use of the composition of any one of claims 1-9 above in the medium or low level disinfection of surfaces used in the food industry.

14. Kit for the *in situ* reconstitution of a disinfectant and/or sporicidal composition according to any one of claims 1 to 9 above, wherein the glutaraldehyde is in a first container, the alcohol and the ammonium compound are in a second container, and the at least one salt is in a third container.

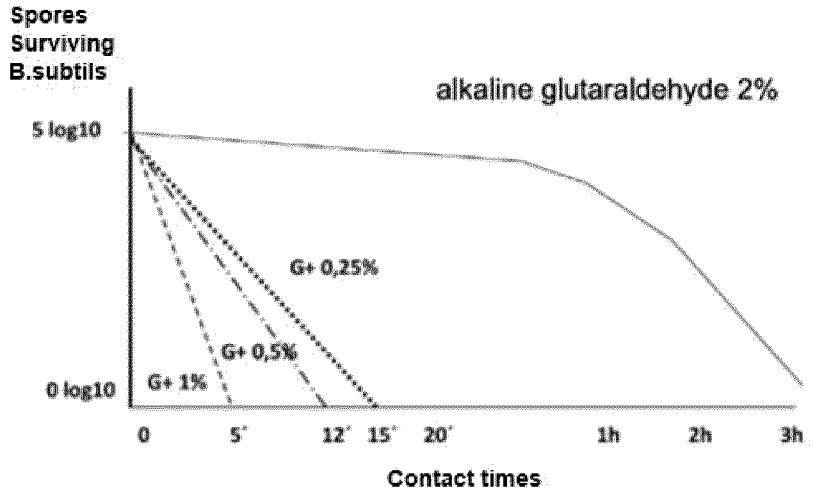
15. Kit for the *in situ* reconstitution of a disinfectant and/or sporicidal composition according to any one of claims 1 to 9 above, wherein the glutaraldehyde and the alcohol are in a first container and the at least one salt and the ammonium compound are in a second container.

16. Kit for the *in situ* reconstitution of a disinfectant and/or sporicidal composition according to any one of claims 1 to 9 above, wherein the glutaraldehyde and the alcohol are in a first container, the at least one salt is in a second container, and the ammonium compound is in the third container.

17. A process for the *in situ* reconstitution of a disinfectant and/or sporicidal composition according to any one of claims 1 to 9 above, comprising the step of providing the glutaraldehyde, alcohol and/or salt in a concentration greater than that required to generate the

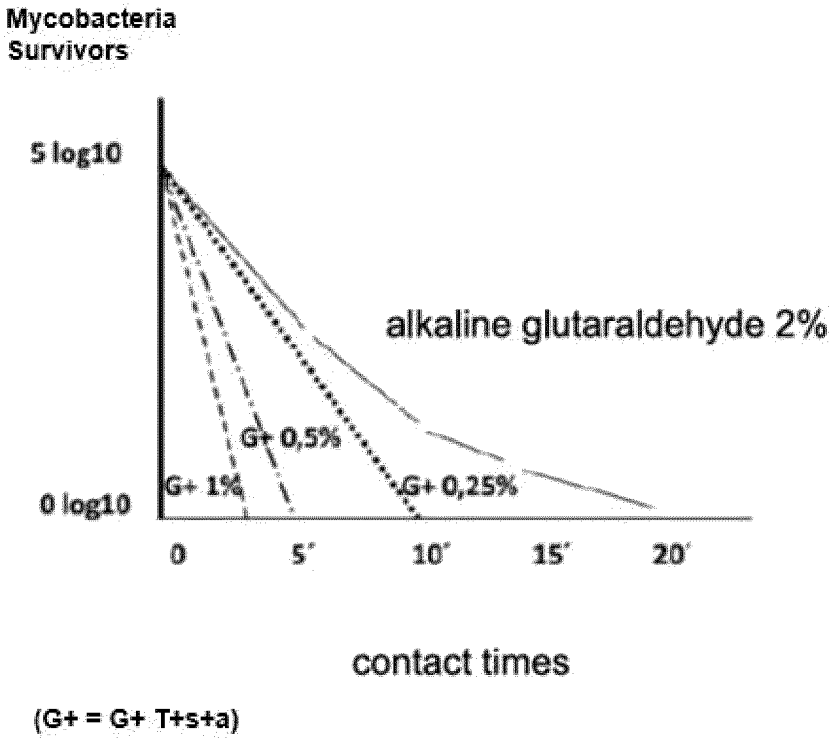
reconstituted composition, and then diluting them *in situ* to the required concentration.

Figure 1: Sporicidal effect of glutaraldehyde at different concentrations between 5 min and 3 hours using the activator Acti1.



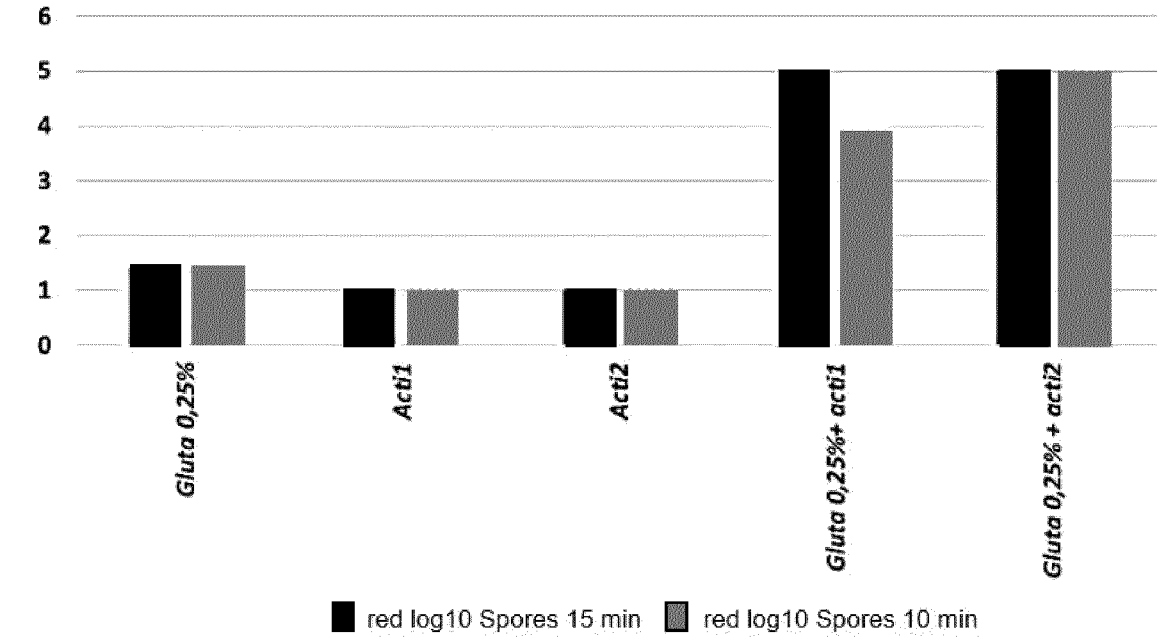
(G+ = G+ T+s+a)

Figure 2: Mycobactericidal effect of glutaraldehyde at different concentrations (Time 0-20) using the Acti1 activator (M fortuitum on rugose portagem).



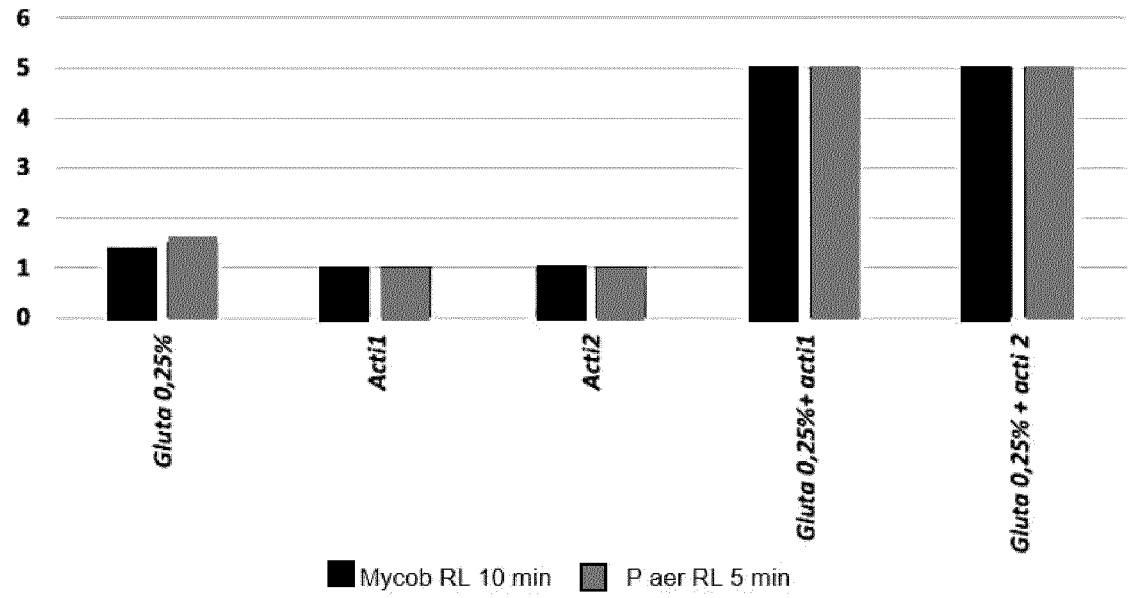
(G+ = G+ T+s+a)

Figure 3: Sporicidal effect (log10 reduction of 3M commercial spores) of Glutaraldehyde 0.25% alone or with two types of activators in 10 or 15 min



Act1= T +s+a; Act2= B+s+a

Figure 4: Bactericidal effect (log10 reduction on rugose germs) of Glutaraldehyde 0.25% alone or with two types of activators in 5 min (P aeruginosa) or 10 minutes (M fortuitum).



Act1= T +s+a; Act2= B+s+a

Figure 5: Sporicidal effect (log10 reduction of 3M commercial spores) of Glutaraldehyde 0.25% alone or with various components of Acti2 in 10 min

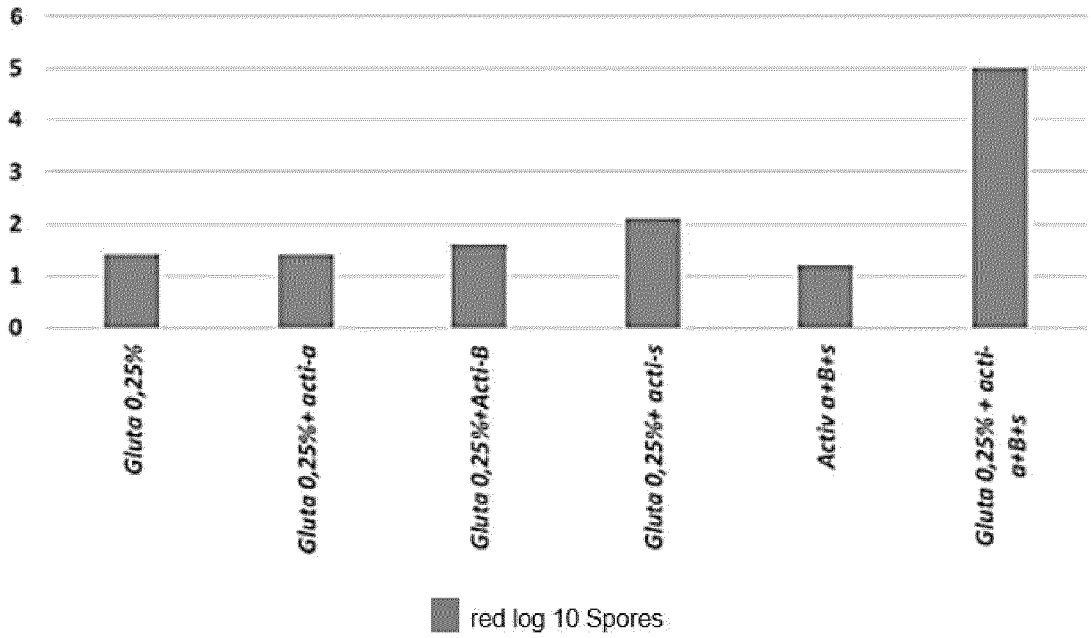
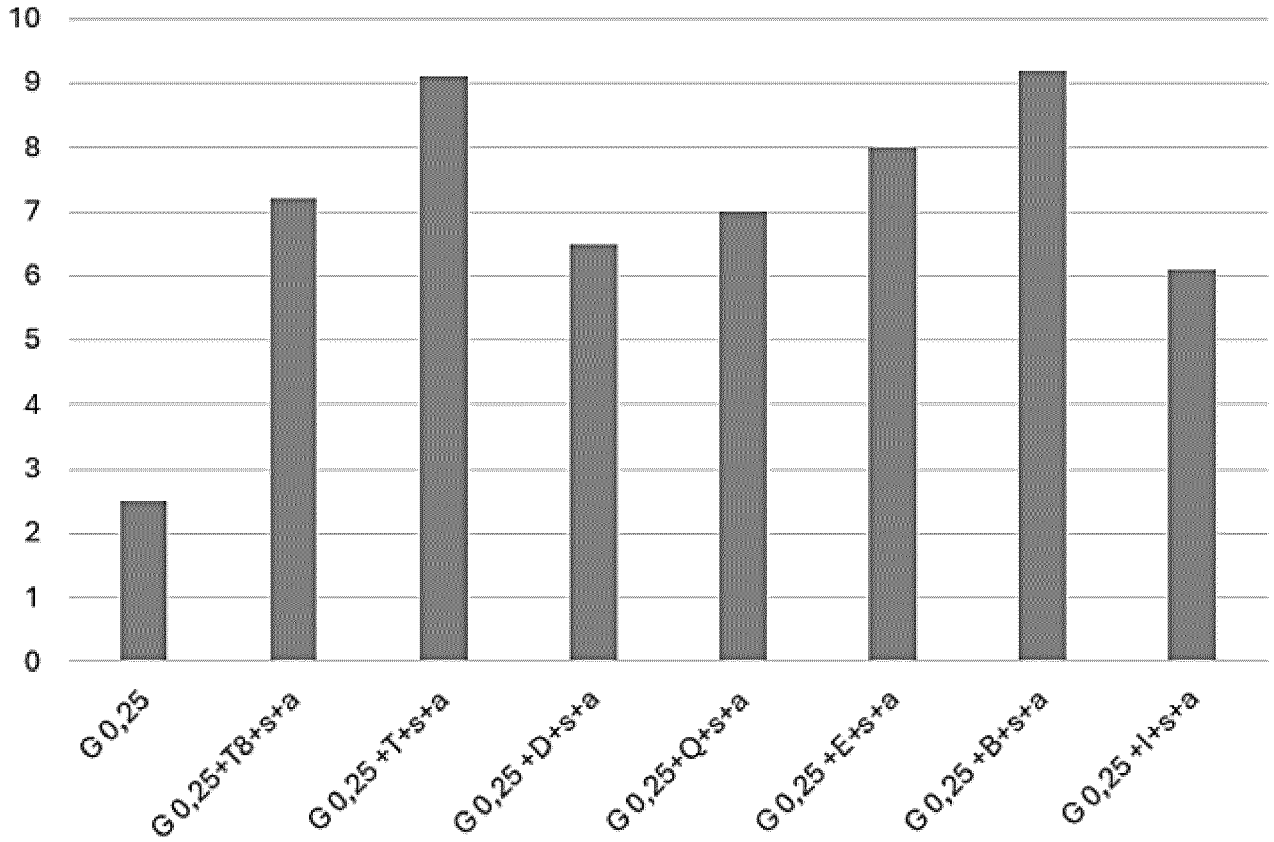


Figure 6: Synthetic score (microbicidal + lack of harm combined effects)



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065658

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N25/02 A01N31/02 A01N33/12 A01N35/02 A01P1/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RU 2 395 962 C1 (FEDERAL NOE G UCHREZHDENIE 48 [RU]) 10 August 2010 (2010-08-10)	1-17
Y	paragraphs 1-31 and 36; the tables; the examples and the claims	1-17
Y	CN 115 530 166 A (HUNAN XIANGNONG ANIMAL PHARMACEUTICAL CO LTD) 30 December 2022 (2022-12-30) the whole document	1-17
Y	CN 106 804 620 A (HECHI TECH DEV CT) 9 June 2017 (2017-06-09) the whole document	1-17

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 September 2024	01/10/2024

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	the whole document	1-17

Y	CN 107 509 740 A (VETERINARY SCIENCE INST OF HEILONGJIANG PROVINCE) 26 December 2017 (2017-12-26) the whole document	1-17

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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CN 107509740 A	26-12-2017	NONE	